

**DETECTION OF OVULATION BY ESTIMATION
OF CHLORIDE CONTENT IN CERVICAL MUGUS**

**THESIS
FOR
MASTER OF SURGERY
(OBSTETRICS & GYNAECOLOGY)**



BUNDELKHAND UNIVERSITY
JHANSI (U.P.)

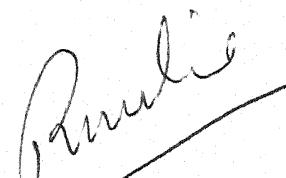
C E R T I F I C A T E

This is to certify that the work entitled "DETECTION OF OVULATION BY ESTIMATION OF CHLORIDE CONTENT IN CERVICAL MUCUS" which is being submitted as a thesis for M.S.(Obstetrics and Gynaecology) by Dr. SATWANT KAUR SALUJA has been carried out in the department of Obstetrics and Gynaecology, M.L.B. Medical College, Jhansi.

She has put in the necessary stay in the department as per university regulations.

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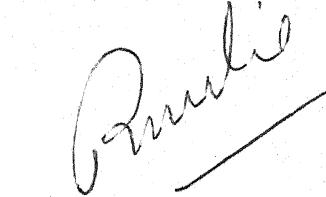
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ACKNOWLEDGEMENT

It is with due regards that I pay my gratitude to my most respected and learned teacher Prof. R. Mitra, M.S., D.G.O., F.A.I.M.S., Head of the department of Obstetrics and Gynaecology, M.L.B. Medical College, Jhansi. She had given me her valuable advice and genial suggestions which played an important role in successful completion of the present work.

I am greatly indebted to my guide Dr. (Mrs.) Sanjaya Sharma, M.D., Assistant Professor, Department of Obstetrics and Gynaecology for her untiring efforts and constant supervision throughout the period of this work. She had guided me at each step and for this I am very thankful to her.

I am thankful to Dr. V.K. Sharma, M.D. (Fath.), Assistant Professor for his patience in elucidating the various aspects of the work carried in this volume. Without his support and knowledge of various cannons of the subject it would have been well high impossible to accomplish the project for me.

I also thanks to all the members of the department of Obstetrics and Gynaecology in particular Dr. (Mrs.) M. Kapoor, M.S., Associate Professor, Dr. (Mrs.) Sunita Arora, M.S., Associate Professor, Dr. (Mrs.) Usha Agarwal, M.S., Associate Professor and Dr. (Mrs.) S. Kharakwal, MD, Assistant Professor for their expert guidance throughout the course of study.

I deeply value the rich advice and assistance given by colleagues and seniors during this study.

I pay my thanks to Shri Phool Chandra Sachan for his timely help in preparing this typed manuscript.

I bow in reference to my lady subjects who have formed the material for present study. Nothing can replace them.

Lastly, I pay my regards and thanks to my parents who helped me a lot throughout the study. Without their help and sincere efforts it would have been impossible for me to continue the study. I would be failing in my duty if I don't express my gratitude to my caring husband. He always inspired me zeal and moral to build sufficient strength in me to complete this work. I sincerely dedicate this work to him. I also pay my thanks to my dearest friend Dr. Uma for her sincere efforts and help rendered by her to complete this project.

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Dated: 1. 8. 91

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I N T R O D U C T I O N

I N T R O D U C T I O N

All gynaecologists are to face the problems of infertile couple. To uncover the causative factors in both partners a well planned search is mandatory. These include examination of semen, evaluation of cervical, endometrial, tubal and ovarian function.

Primary infertility is the inability to achieve pregnancy after at least one year of unprotected coitus. Secondary infertility implies infertility with proven past fertility including ectopic gestation. Recurrent abortions are considered a form of secondary infertility. The goals of infertility evaluation are two folds. To discover the aetiology and to provide a prognosis for future treatment based on the findings.

The incidence of infertility, both primary and secondary varies from 2-10 percent of all married couples. It forms 8-10 percent of cases seen in the gynaecological clinics of Indian hospitals. Primary infertility was seen in two percent and secondary in 3-7 percent cases. As the age increases secondary

infertility was seen to rise mainly due to infections, post abortal, puerperal and STD.

Pregnancy rates in normal nulliparous women is the 50 percent in five months, 75 percent in about 10-12 months, and 100 percent in about 15-18 months. The variations depending on the age of the woman and the fertility of her partner.

A couple who seeks consultation for infertility is already under considerable stress, and the number of tests and visits to the clinic can introduce new stresses and anxieties and interfere with normal life style of the couple. For these reasons, the evaluation should be well planned, objective, accurate and rapid.

Conception results from the fertilization of the ovum by a spermatozoon. In order to understand the powers of fertilization, it is essential to know the limiting period of viability which governs the useful life of a spermatozoon after ejaculation, during which it is capable of penetrating and fertilising the ovum. Though a spermatozoon after ejaculation may

remain motile for a longer period, its useful life span is limited to twenty four hours and that after this short interval, it is incapable of performing its biological duty. The period of survival of a mature ovum is probably even shorter than that of a spermatozoon and the time which elapses after its escape from a ripe graffian follicle. Nevertheless, there are several other parameters which have been used as presumptive evidence for recent ovulation. The presence of a biphasic basal body temperature curve, a shift in the maturation index of vaginal cytology, a change in quantity and quality of the cervical mucus, the appearance of a secretory endometrial biopsy and an increased amount of urinary pregnanediol all constitute the more popular, clinically acceptable, indirect indices of ovulation.

A recent reliable test is the concentration of LH surge. Radio immuno-assay of plasma or urinary LH rise predicts ovulation within 20 to 36 hours. The average being 30 hours (Collins, 1981). Diagnosing LH surge has important therapeutic application in ovulation

induction, artificial insemination and IVF. Pelvic ultrasound allows direct visualisation of the presence, size and number of developing follicles. Follicular diameters ranging from 18-25 mm are considered as mature follicles. A single assay of serum progesterone during the midluteal phase also provides confirmation of presumed ovulation without indicating the actual time it occurred.

Basal body temperature falls at the time of ovulation by half degree Fahrenheit. Subsequently, during the follicle and its entry into fallopian tube during which it is potentially fertilisation, is estimated at twelve hours. The significance of this statement is that coitus, to be capable of fertilisation, must take place within twelve hours of ovulation. So unless, intercourse occurs frequently enough to cover the short time during which the ovum is viable, it may be months or years, before a chance encounter achieves successful fertilisation.

Knaus concluded that ovulation most commonly occurs fourteen days before the onset of the next

period, no matter how short or how long the inter-menstrual interval.

The obvious significance in the detection and timing of ovulation is in the management of a case of infertile couple and greater importance of applying to the problem of fertility control.

Ovulation is a very important phenomenon in the reproductive life of a woman. Infertility requires the detection of this event to determine sexual behaviour that will preclude pregnancy.

The only positive evidence that ovulation has occurred in the human being is the recovery of an ovum or the occurrence of pregnancy. Pregestational half of the cycle the temperature is slightly raised above the pre-ovulatory level, and the rise is of the order of half to one degree. This phenomenon is due to the slight pyrogenic action of progesterone and is therefore, presumptive evidence of the presence of a functioning corpus luteum and hence ovulation.

A scope preparation obtained from the upper lateral vaginal wall and examined under the microscope

after staining should show cytological evidence of corpus luteum activity of taken any time after ovulation has occurred and before the next period is due. The cells are predominantly of the basophilic intermediate type with vesicular nuclei. They show edge curling and folding. The so called envelop effect, and are clustered together. Cytology can thus provide useful evidence that ovulation has occurred.

The cervix plays a significant role in reproduction. Cervical mucus represents the first barrier spermatozoa have to face along their path. Hence a careful study of the cervical mucus is an essential part of infertility investigation. Apart from its value in assessing ovarian function, it may also unearth other causes for infertility such as genital infection and immunologic incompatibility.

Cervical mucus displays cyclical activity which is maximal at ovulation to facilitate migration, capacitation and the storage of spermatozoa in the cervical crypts. Many analyses, although useful, cannot routinely monitor the fertility period and establish probable ovulation dates.

The cervical mucus has been used in the study of ovarian function in many ways. The physical characteristics studied were volume, pH, viscosity, spinnbarkeit, arborization and cellularity. The chemical characteristics included estimation of chloride content sugar, total proteins and urea.

Volume of cervical mucus gradually increased to its maximum from immediate postmenstrual to mid-menstrual period and again diminished in premenstrual period.

Viscosity was found to be thick in post-menstrual and premenstrual phases and thin in mid-menstrual phase.

The glandular elements of the cervix proliferate during the follicular phase and epithelial cells became taller. Under the influence of oestrogen the glands also actively secrete a mucus which will stretch into threads measuring more than 6.5 cm, and even 10-15 cm at the time of ovulation. This property of spinnbarkeit is the basis of the thread test for oestrogen in circulation. During the luteal phase the cervical

glands become more branched and their secretion changes its physical and chemical properties. The mucus becomes more viscous and forms a more secure cervical plug. It loses its ability to stretch without breaking and resists penetration by spermatozoa. These changes are brought about by progesterone and are related to an increase in the amount of protein in the mucus and to the presence of phospholipids.

During the follicular phase the cervical mucus absorbs water and salts and when allowed to dry, deposits crystals of sodium chloride and potassium chloride in a characteristic pattern which suggests the fronds of fern. At the time of ovulation the secretion is so profuse that it may be noticeable as a vaginal discharge. The ovulation cascade. Its special character at this time makes for its easy penetration by spermatozoa. This property is related to its low content of protein. Campos da Paz reported that cervical mucus was unfavourable to sperm penetration unless it gave the fern reaction. Progesterone reduces the electrolyte content of the mucus, so by the twenty second day of the cycle its property of fanning or arborization on drying is lost.

Zondek and Rozin presented evidence to indicate that whereas in a normal pregnancy there is no fern reaction, the presence of the fern pattern in a pregnancy would suggest corpus luteum or placental insufficiency and patient might have an abortion.

Since it has been demonstrated that sodium chloride is necessary for a positive fern test. Keeping this in mind an attempt has been made to evaluate variations of chloride level in cervical mucus during the menstrual cycle and its utility in predicting ovulation. Chloride content in cervical mucus can be detected either by semiquantitative test known as spot test or by quantitative test known as Varley test. This is done by titration method.

Lastly, all results were compared with endometrial biopsy report, taken in premenstrual phase. So our studies have shown that ovarian function can be related to alteration in chloride levels in cervical mucus.

REVIEW OF LITERATURE

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Infertility implies apparent failure of a couple to conceive, while sterility indicates absolute inability to conceive, for one or more reasons. If a couple fails to achieve pregnancy after one year of unprotected intercourse, it is an indication to investigate the couple. This is based on observation that pregnancy rates in normal nulliparous women is 50% in five months, 75% in about 10-12 months and 100% in about 15-18 months the variations depending upon the age of the woman and the fertility or her partner (Devi et al, 1989).

According to a recent multicentric study although 16% women became pregnant during the period of study, the female causes were seen in only 34%, male factors 13%, both in 24%. In the female no demonstrable cause was noted in 31% but 27% had tubal obstruction or pelvic adhesions, 15% acquired tubal abnormality, 12% anovulatory cycles, 10% endometriosis and 7% had hyperprolactinaemia (Cates et al, 1985).

Conception results from fertilization of ovum by a spermatozoon. It is now generally accepted that

though a spermatozoon after ejaculation may remain motile for a longer period, its useful life span is limited to twenty four hours and that, after this short interval. It is less capable or performing its biological duty. The period of survival of a mature ovum is probably even shorter than that of a spermatozoon and the time which elapses after its escape from a graffian follicle and its entry into the fallopian tube during which it is potentially fertilizable, is estimated at twelve hours and rarely upto 24 hours. The significance of this statement is that coitus, to be capable of fertilization, must take place in the 24 hours period around ovulation. According to this, unless intercourse occurs frequently enough to cover the short time during which the ovum is viable, there is no conception. Ovulation most commonly occurs fourteen days before the onset of next period though variations are known (Padubidri and Daftary, 1989).

The obvious significance in the detection and timing of ovulation is in the management of a case of infertile couple and greater importance for applying to the problem of fertility of control. Many laboratory methods

for detection and diagnosis of ovulation have been devised. Still this important basic phenomenon remains retrospective and numerous approaches have been realised to attest to its probable occurrence. The absolute confirmation of ovulation, though of profound biological significance has to be done by use of indirect means for want of simple direct one to establish such temporal relationship. Many analyses although useful, cannot routinely monitor the fertility period and establish probable ovulation dates (Shastrakar et al, 1980).

To date the most frequently tests for ovulatory function are the basal body temperature chart, vaginal smear, endometrial biopsy, bioassay of the excretion of hormones in urine, single luteal phase serum progesterone assay, studies of physical and chemical characteristics in different phases of the cervical mucus (Sweemey et al, 1969).

The cervical mucus has been used in the study of ovarian function in many ways. The physical characteristics studied were volume, pH, viscosity, spinnbarkeit, arborisation (Palmleaf test) and cellularity. The chemical characteristics included estimation of sugar, total proteins, urea and chloride content. Apart from these characteristics

cervical mucus can be used to detect ovulation by changing its colour from white to light brown on heating. This is caramel test and is related to ovulatory phenomenon (Campos Da Paz, 1974).

It is established that the basal body temperature falls at the time of ovulation by about half degree Fahrenheit. Subsequently, during the pregestational half of the cycle the temperature is slightly raised above, the pre-ovulatory level and the rise is of the order of half to one degree. More over if the patient conceives, the temperature remains at this level and does not fall as it would at the onset of menstruation. This phenomenon is due to the slight thermogenic action of progesterone and is therefore, presumptive evidence of presence of functioning corpus luteum and hence ovulation (Perlman and Israel, 1975).

Rothchild and Barnes stated that the thermogenic effect of progesterone may be mediated through a hypothalamic centre.

Some workers suggested that basic mechanism for thermogenesis is Nor epinephrine (NE) and not progesterone. They stated that pre-ovulatory NE ranged from 7.5 to 18.3

ug/24 hours in urine, whereas postovulatory NE was significantly elevated and ranged from 14.7 to 51.6 ug/24 hours. Mean values of epinephrine(E) did not differ before and after ovulation. It is postulated that progesterone causes an increased excretion and production of NE, which is responsible for the increased thermogenesis after ovulation (Frederick P. Zuspan and Padma Rao, 1973).

The regulation and control of body temperature is complex and involves many control systems with both positive and negative feedback mechanisms. The hypothalamus synchronizes the outflow of the autonomic nervous system, especially the sympathico adrenomedullary system with interplay of all neuroendocrine systems and their target glands. The interplay of these systems controls heat gain and loss. The chemical regulation of heat production may be mediated through the release of NE from the sympathetic nerve endings. The increase in NE excretion was 1 $\frac{1}{2}$ to 3 fold during post ovulatory period as compared to the pre-ovulatory period. The increase in NE excretion during the post-ovulatory phase explains the action of progesterone during that period. In an ovulatory

cycles neither significant increase in NE excretion nor a corresponding elevation in temperature was observed. In fact there is interaction of the ovarian hormones in the excretion and storage of the neurotransmitter NE. Estrogen promotes a steady excretion of NE and also enhances an increase of NE storage in nerve vesicles. Ovulation results in an increase production of progesterone which in turn unlocks and releases the stored NE. The increased circulation and excretion of NE act on hypothalamic heat controlling centre to increase the basal body temperature. Hence, the basic mechanism of change in basal body temperature is NE and not progesterone (Karlberg et al., 1962).

Colpo-cytology have always had a definite place in the management of infertility cases. Although recent advances in biochemistry have made hormone assays freely available for endocrine studies but colpo-cytology serve as the screening procedure and will enable one to select cases which may require sophisticated hormone assay. For colpo-cytology lateral vaginal wall scrapes were taken and cytology smears were fixed in equal parts of alcohol and ether and stained by papanicoleau stain. Because of

hormonal effect the number of different types of cells differ in different phases of menstrual cycle.

Cells which are because of oestrogenic effect are mature epithelial eosinophilic cells with pyknotic nuclei, while cells of progesterone effect are predominantly basophilic with intermediate celis having vesicular nuclei.

De Allense (1950), Arronet and Turnbul (1957) and Punpel (1965) divide the menstrual cycle into seven phases.

Phase I : Menstrual phase.

Phase II : Post menstrual phase.

Phase III : Pre-ovulatory phase.

Phase IV : Ovulatory phase.

Phase V : Post-ovulatory phase.

Phase VI : Luteal phase.

Phase VII : Pre-menstrual phase.

In colpo-cytology studies, phases II and III correspond to the proliferative phase and phases V to VII correspond to secretory phase of ovulation occurred. The number of mature eosinophilic cells per 100 cells counted is expressed as the Karyo Pyknotic index. This is high

in the early part of menstrual cycle that is in phases II and III while falls after ovulation i.e. in phases V to VII. The peak values of K.I. in the first half of menstrual cycles vary significantly. It may varied from 25 to 75 (Saraiya et al, 1977).

Luteal phase deficiency is inadequate progesterone. Production by the corpus luteum, there are significance number of unexplained infertility due to this cause (Jones, 1972).

Pundel (1962) stated that it is possible to utilise the vaginal smear as a qualitative test of luteal function.

Luz (1962) described two cytohormonal patterns characteristic of disturbed luteal function. One is the incomplete neutralisation of the estrogen effect of first half of cycle, second is an initial fall in K.I. followed by an irregular pattern of waves. Place of endometrial cytology in detection of endometrial malignancy is well known but there is also a definite place of endometrial aspiration in detection of ovulation. Diagnostic accuracy of endometrial aspiration done with ISAAC's cell sampler

was compared with endometrial biopsy and dilatation and curettage (Vithal N. Purandare, 1982).

An endometrial aspiration was taken just before performing endometrial biopsy during premenstrual phase.

Endometrial biopsy was carried out with the ISAAC cell sampler, a flexible metal canula 1.9 mm in diameter which can be inserted upto 7 cm into the endometrial cavity. It contains multiple holes for sampling. A cervical shield seals the cervical os to help to create a vaccum for aspiration of endometrial material and also prevent cellular contamination from the cervix. A plastic sheath covers the canula during insertion and withdrawal to prevent further contamination. A regular syringe is attached to the end of the cannula for aspiration. The aspiration material is smeared on the clear glass slide which is immissed in fixative and stained by papanicolaou technique.

Endometrial cells in secretory phase can be identified easily by a large distinct secretory vacuole occupying almost all the cytoplasm distorting the neucleus. This activity seen only after ovulation. While the proliferative type cells are closely packed and have a well

presented, round, hyperchromatic nucleus and very little cytoplasm. Hence where such cells are seen predominantly in cytology one can conclude that the cycle was an ovulatory (Peters et al, 1958; and Grubb, 1977).

By using the ISAAC cell sampler, this was found a correlation of 95% between the histologic and cytologic diagnosis (Hutton et al, 1978).

Zubair et al (1981) had an accuracy of 92% in detection of ovulation by endometrial aspiration.

Though it is not always possible to pin point ovulation with this method, the aspiration can be repeated in many cycles.

The sampling method has a considerable influence on the appearance of the endometrial cells. The cells which are spontaneously desquamated from the endometrium into cervical or vaginal secretions are quite different from those removed forcibly. These retain their columnar configuration while the desquamated cells tend to obey the laws of surface tension and assume a spherical form.

The aspiration is to be done in midluteal phase i.e. approximately 21st day onward of a 28 day cycle

because plasma progesterone activity is at its peak about 8-9 days after ovulation. Endometrial columner cells will show maximum morphological changes at this time (Bhagwat and Jiwane, 1983).

There is a true lapse between the response of deeper endometrial glands and superficial epithelium of endometrium. They epithelium of endometrium and deeper glands are anatomically two different structures and hence materials from those two i.e. endometrial aspiration and endometrial biopsy respectively can hardly be compared. There is a lag in the secretory transformation of surface epithelium during the first half of luteal phase. In the mean time however, the underlying glandular epithelium shows all the changes of luteal phase (Ludinghausen and Anastasiadis, 1984).

The incidence of tuberculosis in India is comparatively higher than the other parts of the world. Since endometrial tuberculosis and an ovulation are so closely associated with sterility so that endometrial biopsy become are important investigation of infertility to exclude tuberculosis and to confirm ovulation (Gupta, 1979).

The incidence of anovulation in infertility as reported by various authors is variable between 5 to 36% (Sharman, 1943; Stallworthy, 1948; Sachdeva, 1959; Saha, 1961 and Israel, 1967).

By studies of endometrial biopsy persistant anovulatory cycles found on repetitive studies in 52.8% (Wong, 1950), while other workers found only in 10% (Israel, 1967). Thus the value of initial endometrial biopsy remains uncertain in establishing the diagnosis of repetitive anovulation.

There was poor chance of having a baby if the initial biopsy has shown anovulation or atrophic endometrium (Stevenson, 1965).

Such patients who are unable to develop an adequate secretory response may be helped by hormonal therapy (De Moraes-Ruehsen et al, 1969).

The higher incidence of genital tuberculosis in secondary infertility group emphasizes that search for tuberculosis is mandatory when puerperal and post abortal sepsis do not respond to usual antibiotics (Malkani, 1980).

Single luteal phase serum progesterone assay can be used as an indicator of ovulation because there is striking increase in blood progesterone levels in the luteal phase of ovulatory cycles.

The level of serum progesterone during the follicular phase was 1-2 ng/ml. Progesterone levels rose in the luteal phase beginning 1 or 2 days after LH peak to reach a peak concentration ranging from 15-30 ng/ml. A low level of plasma progesterone below 5 ng/ml at mid luteal phase, suggests corpus luteal phase defect.

A single serum sample obtained in the mid luteal phase between 11 and 4 days prior to subsequent menses, with a progesterone concentration of greater than 3 ng/ml, was always accompanied by a secretory endometrium. Therefore the finding of progesterone levels greater than 3 ng/ml in this period provides the clinician with presumptive evidence that ovulation has occurred in that cycle.

The endometrial biopsy provides a more consistent indication of previous ovulation as the secretory pattern persists until menstruation begins, while serum

progesterone level falls to follicular phase values 3 to 4 days prior to menstruation (Robert Israel et al, 1971).

The cervix plays a significant role in reproduction. Cervical mucus represents the first barrier spermatozoa have to face along their path. Hence a careful study of the cervical mucus is an essential part of infertility investigation (Shammi Mehra et al).

Cervical mucus displays cyclical activity which is maximal at ovulation to facilitate migration, capacitation and the storage of spermatozoa in the cervical crypts. During the periovulatory period the viscosity of cervical mucus drops, spinnbarkeit increases and characteristic fern patterns appear upon air drying. These changes which are conductive to penetration by spermatozoa are accompanied by an increase in water content, increased concentration of certain electrolyte particularly sodium and chloride and alteration in the quality of proteins present.

The importance of the cervical factor in an infertility evaluation is highlighted and found that cervical factor responsible in one third to one half of patients (Mazer and Israel, 1959).

Irving (1939) found out pouring of cervical mucus at ovulation. Volume of cervical mucus is maximum at mid cycles to aid sperm migration. Usually in fertile women it ranged from 0.4 to 0.7 ml and when it fell below 0.4 ml the post coital test was unfavourable in infertile group (Moghissi and Neuhaus, 1966; Harvey et al, 1960).

Sperm motility is known to be arrested in acidic medium. Normally in fertile women pH of cervical mucus is 7.2 - 7.6, where the pH fell below 7, it was always associated with an unfavourable postcoital test.

Spinnbarkeit gradually increased in length from postmenstrual phase to maximum of 6-20 cm in midmenstrual phase and then gradually diminished in premenstrual phase. Spinnbarkeit was maximum when mucus was clear. This and copioes with positive Palmleaf reaction and relative acellularity (Chitra Lekha Sarin, 1970).

From most reported series spinnbarkeit at midcycle exceeds 10 cm for a good or excellent. Post coital test (Gibbon and Mathur, 1966 and Mitra et al, 1975).

Papaniclaou observed that cervical mucus when allowed to dry on a glass slide formed a leafy pattern.

In addition, crystalline fern formation in the cervical mucus at ovulation and very little or none just before the menstrual flow has been noted. This reaction is known as Palmleaf reaction or fern test (Mcsweeney and Sbarra, 1964). Palmleaf (Arborizations) was graded as follows :

Negative : No P.L. reaction.

Atypical : P.L. reaction in atypical pattern.

+ Sparse P.L. reaction.

++ P.L. reaction spread even most of slides.

+++ Dense P.L. reaction.

The fern reaction was found to be absent during pregnancy and at the menopause. By administering oestrogen to menopausal women the fern pattern was able to appear.

Compos DA Paz reported that cervical mucus was unfavourable to sperm penetration unless it gave the fern reactions.

Zondek and Rozin presented evidence to indicate that whereas in a normal pregnancy there is no fern reaction the presence of the fern pattern in a pregnancy would suggest corpus luteum or placental insufficiency and the patient might have an abortion.

Fern test is generally accepted as a useful laboratory procedure for the determination of ovarian function.

Certain rheologic properties of cervical mucus are known to change during the menstrual cycle. The protein components might be expected to exhibit similar variations. The albumin, globulins and nonmigrating fraction (NMF) believed to be the indigenous mucoid proteins of cervical mucus.

Immediately prior to the time of ovulation or co-incident with this time, there was a marked and significant decrease in the amount of albumin in the cervical mucus. This decrease in albumin was associated with concomitant increase on the NMF, while the amount of total globulins remained fairly constant. Within two or three days after ovulation, a reversal occurred during which the albumin increased, sometimes far above its initial value and NMF decreased. The globulin content of cervical mucus did not change significantly throughout the menstrual cycle.

The reciprocal changes in the albumin and NMF are apparently the result of normal cyclic release of

oestrogen and progesterone and seem to be associated with ovulation. This conclusion is plausible to view of the demonstration absence of protein changes in the mucus of women receiving oral contraceptives (Progestin, estrogen combination) throughout the menstrual cycle. It is conceivable that the preovulatory decrease of albumin or the increase of NMF may be utilise to determine the onset of ovulation (Moghissi, 1966).

If there is an appreciable fall in sugars and rise in protein at mid cycle then cervical mucus is said to be hostile for penetration of sperm. The decreased sugar levels at mid cycle interfere with nutrition and energy supply for sperm motility and transport, while the increase protein content contributes towards increased viscosity hindering sperm penetration and explaining, the unfavourable postcoital test (Birenberg et al, 1963 and Mastroianni & Lemert, 1974).

The caramel test can be use to detect ovulation. It involves heating of cervical mucus and changing of the white colour to light brown that is more significant in relation to ovulatory phenomenon (Campos Da Paz, 1974).

The white appearance of cervical mucus was always associated with typical fern pattern and secretory phase of the endometrial biopsy about 13^m to 14^m day of the cycle thereafter the cervical mucus showed change over of colour from white to sight brown around 15th day. From 16th day onwards the brown colour of cervical mucus was associated with a typical fern pattern like pre-ovulatory phase with secretory endometrium (Engineer et al, 1968 and Sarin, 1971).

White appearance of cervical mucus and a typical fern pattern is related to oestrogenic stimulation and turning of the white to light brown or caromel in fertile period, the most earliest sign seen on cervical mucus may reflect follicular rupture, corpus luteum formation and fall in plasma oestrogen levels. A positive caromel test following the white colour strongly suggest ovulation (Shastry et al, 1950).

Changes in cervical mucus which are conductive to penetration by spermatozoa are accompanied by increase in water content, increased concentration of certain electrolytes particularly sodium and chloride. So variations in chloride level in cervical mucus during the

menstrual cycle can be used in predicting ovulation because ovarian function could be related to alteration in chloride levels in the cervical mucus.

Crystalline fern formation in the cervical mucus at ovulation and very little or none just before the menstrual flow has been noted. It was deduced that these crystals were sodium chloride and that the fern reaction required the concomitant presence of sodium chloride with mucin like substances (Lander strom-Lang).

Some workers found crystalline fern formation similar to that in cervical mucus, where a solution of native egg albumin in aqueous 0.9 percent sodium chloride dried on a slide (Rypberg, 1948).

It was also found that the sodium chloride concentration in the cervical mucus ash at or near ovulation is almost 97% of the total salts.

To determine the chloride changes in cervical mucus estimations of chloride done in pre, during and post ovulatory phases. Chloride levels can be determined, either semiquantitatively or quantitatively. Semi quan. test for chloride anion can be done by using spot test, while quantitatively it can measured by Varley test. In throughout the cycle, we observe that the level of chloride

both types silver nitrate is used as a salt and potassium chromate as an indicator.

The results of the spot test were noted by the intensity of the spot or stain on the test paper ranged from 0.1% in the early proliferative phase to a higher value of about 0.9% at the presumable time of ovulation. The day after ovulation, when the corpus luteum is producing progesterone there is a decided change in spot intensity with a drop to below 0.5% in the chloride concentration in the cervical mucus, when this drop has occurred we assume that ovulation has taken place. This drop in sodium chloride concentration occurs simultaneously with the rise in temperature when using the temperature chart. For two or three days prior to the drop in sodium chloride, the spot test may show an intense stain. This indicates that the cervical mucus is then receptive to the sperm and ovulation has occurred or is occurring ovulation takes place on the day or days of highest concentration chloride. The day of probable ovulation as determined by this test was found usually to occur between 13 to 15 days before the anticipated menses regardless of the length of the cycle. If there is little or no change in the spot test throughout the cycle, we assume there has been no

ovulation (Zondek and Rozin, 1959; McSweeney and Sbarra 1964).

On many different occasions, some workers performed chloride or spot tests on nasal mucus along with cervical mucus. Identical cyclic variations were noted with each secretions. However, a considerable less spot intensity (Chloride concentration) was noted with the nasal mucus. The use of nasal mucus in conjunction with cervical mucus could be highly desirable and studies of this and related problems are in progress.

The maximum level of sodium (107 to 196 m mol/gm) occurred on the day of ovulation and was usually preceded by a surge from a relatively low value 1 to 2 days prior to ovulation (Hardy et al., 1970).

Some workers found sodium chloride level to be constant at 100 to 150 m mol/g throughout the cycle. However in dry residue there were cyclical variations in the level with very sharp rise during the mid cycle (Singh and Boss, 1973).

It has been found that chloride levels to be below 100 m eq/l between the 7^m to 12^m day and after

17^m day of menstrual cycle in majority of cases. Between 13^m to 16^m day of cycle i.e. on presumed days of ovulation the levels were above 100 m eq/l in ovulatory cycles. However, it was low in anovulatory cycles (Mathur and Dayal, 1987).

The accuracy of chloride spot test and fern test with endometrial biopsy was compared. They found the chloride spot test to be 94% accurate and Fern test 97.82%.

Roland (1952), Zondek and Rozin (1954) detected the accuracy of cervical mucus fern test to be 100%, 85% and 89.9% respectively as compared to endometrial biopsy.

MATERIAL AND METHODS

MATERIAL AND METHODS

The present study has been carried out on patients with infertility attending O.P.D. of Obstetrics and Gynaecology, M.L.B. Medical College, Hospital, Jhansi.

SELECTION OF CASES

Two groups of patients were registered for study after informed consent.

1. Study group.
2. Control group.

Study group

This group included the cases of primary infertility with following criteria :

- a. Age between 18-35 years.
- b. Regular menstrual cycles.
- c. Period of infertility one year or more.
- d. Clinical findings essentially normal.

Control group

This group included the fertile cases of same age group with no clinically detectable pelvic pathology.

METHOD

All the patients were subjected to following procedure :

- Cervical mucus was collected by a glass pipette with bulb suction three times in a same cycle viz :
 - a. Once between 7th to 12th day of cycle i.e. in preovulatory phase.
 - b. 13th to 16th day of cycle i.e. in ovulatory phase.
 - c. 17th to 22nd day of cycle i.e. in post ovulatory phase.
- Mucus sample was divided into two parts :
 1. First part was spreaded on a dry glass slide and with the help of other glass slide it stretched upto its breaking point. This is Spinnbarkeit or thread test.
 2. Second part was tested for levels of chloride by titration (Varley test, 1967).

It is possible to titrate most cervical mucus directly with an appropriate silver nitrate solution using potassium chromate as indicator.

Reagents

a. Silver nitrate solution 30 m eq/5.10 gm)/l, check at intervals against a standard sodium chloride solution containing 180 m eq(10.53 gm)/l.
1 ml of this should require 6 ml of silver nitrate solution.

b. Potassium chromate 10% solution.

Technique

- Pipette 1 ml of cervical mucus into 6 x 1 inch test tube containing 2-3 ml of distilled water.
- Add 2-3 drops of potassium chromate and titrate with silver nitrate to usual faint brick red colour.

Calculation

$$\begin{aligned} \text{Chloride in cervical mucus(m eq/l)} &= \\ &= \text{ml of Ag NO}_3 \text{ solution} \times 30 \end{aligned}$$

Endometrial Biopsy

In all cases premenstrual endometrial biopsy was undertaken in the same cycle for confirmation of ovulation. All the findings were recorded on a working proforma for further evaluation.

O B S E R V A T I O N S

O B S E R V A T I O N S

In the present study we have studied chloride levels in cervical mucus along with spinnbarkeit test to detect the ovulation in infertile and fertile patients. Then we have compared the chloride levels in cervical mucus with endometrial biopsy.

For study, patients had visited the department in three times in the same cycle. Firstly they came in between 7^m-12^m day of the cycle i.e. in pre-ovulatory phase, secondly in between 13^m-16^m day of cycle i.e. in ovulatory phase and lastly in between 16^m-27^m day of cycle i.e. in post ovulatory phase. In each visit her cervical mucus was collected and divided into two parts. By first part length of cervical mucus on stretching was measured in centimeters. This was Spinnbarkeit test.

By second part chloride content was measured in m eq/l by Varley test.

In last visit endometrial biopsy of the patient had also been taken and subjected it for histopathological examination.

The detection of ovulation was studied by estimating chloride content in cervical mucus and compared with endometrial biopsy. The observed results are mentioned in the form of various tables.

TABLE I

Age-wise distribution of patients.
(Infertile series)

Sl. No.	Age (years)	No. of cases	Percentage
1.	15 - 20	6	15.00
2.	21 - 26	19	47.50
3.	27 - 32	13	32.50
4.	33 - 38	2	5.00
Total		40	100.00

Table I shows the age wise distribution of total patients studied in infertile group. Maximum cases 19 (47.50%) out of 40 were in the age group of 21-26 years followed by 13(32.50%) cases in the age range of 27-32 years. Six(15%) cases were in age group of 15-20 years. Only 2(5%) cases were in 33-38 years of age group.

TABLE II

Infertility periodwise distribution
of cases.

Sl. No.	Period of infertility (years)	No.of cases	Percentage
1.	2 - 4	19	47.50
2.	4 - 6	8	20.00
3.	6 - 8	6	15.00
4.	8 - 10	6	15.00
5.	10 - 12	1	2.50
TOTAL		40	100.00

From the table II, it is evident that maximum number of cases i.e. 19(47.50%) fallen in 2-4 years of infertility period while 8(20%) patients were in 4-6 years infertility group. Infertility period of 6-8 years and 8-10 years had 6(15%) cases each. Only one (2.5%) case had very long infertility period i.e. 10-12 years.

TABLE III

Length of Spinnbarkeit(S.B.) in cervical mucus in the ovulatory phase(infertile group).

Sl. No.	Length of S.B. (cms.)	No.of c cases	Percentage
1.	0 - 2	4	10.00
2.	2 - 4	24	60.00
3.	4 - 6	12	30.00
4.	6 - 8	-	-
TOTAL		40	100.00

It is evident from the table III that the length of Spinnbarkeit in cervical in pre-ovulatory phase was 2-4 cm in 24(60%) cases followed by 4-6 cm in 12(30%) cases. The length of spinnbarkeit was 0-2 cm only in 4(10%) cases.

TABLE IV

Length of Spinnbarkeit(SB) in cervical mucus
in the ovulatory phase (infertile group).

Sl. No.	Length of S.B. (cms.)	No.of cases	Percentage
1.	6- 8	6	15.00
2.	8 - 10	18	45.00
3.	10 - 12	14	35.00
4.	12 - 14	2	5.00
5.	14 - 16	-	-
TOTAL		40	100.00

Table IV shows spinnbarkeit in ovulatory phase. 18(45%) cases had spinnbarkeit length in 8-10 cms range followed by 14(35%) cases who had spinnbarkeit in 10-12 cms range. Six(15%) cases had spinnbarkeit in 6-8 cm range. Only 2(5%) cases had spinnbarkeit in 12-14 cm range.

TABLE V

Length of Spinnbarkeit(SB) in cervical mucus in post ovulatory phase(infertile group).

Sl. No.	Length of S.B. (cms.)	No.of cases	Percentage
1.	0 - 2	6	15.00
2.	2 - 4	18	45.00
3.	4 - 6	9	22.50
4.	6 - 8	6	15.00
5.	8 - 10	1	2.50
TOTAL		40	100.00

Table V shows spinnbarkeit in post ovulatory phase. 18(45%) cases had spinnbarkeit length in 2-4 cm range, followed by 9(22.50%) cases having 4-6 cm range. Six (15%) cases fallen in both 0-2 cms and 6-8 cms range while only one (2.5%) case had spinnbarkeit in range of 8-10 cms.

TABLE VI

Chloride content in cervical mucus in
infertile cases in preovulatory phase.

Sl. No.	Chloride content (m eq/l)	No.of cases	Percentage
1.	0 - 50	3	7.50
2.	50 - 100	33	82.50
3.	100 - 150	4	10.00
4.	150 - 200	-	-
TOTAL		40	100.00

Table VI indicates that 33(82.50%) cases had chloride content in range of 50-100 m eq/l in cervical mucus during pre-ovulatory phase from 7^m-12^m day of cycle, followed by 4(10%) cases in range of 100-150 m eq/l. Only 3(7.5%) cases were having chloride content in 0-50 m eq/l range.

TABLE VII

CHLORIDE CONTENT IN CERVICAL MUCUS IN
OVULATORY PHASE (INFERTILE PATIENTS).

Sl. No.	Chloride content (m eq/l)	No.of cases	Percentage
1.	0 - 50	-	00.00
2.	50 - 100	2	5.00
3.	100 - 150	26	65.00
4.	150 - 200	12	30.00
TOTAL		40	100.00

Table VII shows the chloride content in cervical mucus during ovulatory phase. Maximum 26(65%) cases were having chloride content in range of 100-150 m eq/l followed by 12 (30%) cases having chloride content in the range of 150-200 m eq/l. Only 2 (5%) cases had chloride content in the range of 50-100 m eq/l.

TABLE VIII

CHLORIDE content in cervical mucus in
post ovulatory phase (Infertile cases).

Sl. No.	Chloride content (m eq/l)	No.of cases	Percentage
1.	0 - 50	8	20.00
2.	50 - 100	24	60.00
3.	100 - 150	8	20.00
4.	150 - 200	-	-
	TOTAL	40	100.00

Table VII states about chloride content in cervical mucus in post ovulatory phase i.e. in between 16^m-22^m day of cycle. From the above table, it is evident that maximum 24(60%) patients were having chloride content in the range of 50-100 m eq/l while 8(20%) cases fallen in both 0-50 and 100-150 m eq/l group.

TABLE IX

Endometrial biopsy taken in post ovulatory phase(Infertile cases).

Sl. No.	Endometrial biopsy	No.of cases	Percentage
1.	Secretary	37	92.50
2.	Proliferative	3	7.50
	TOTAL	40	100.00

From the table IX it is evident that 37(92.5%) patients had secretary type of endometrial biopsy taken in post ovulatory phase but 3(7.5%) cases had proliferative type of endometrial biopsy.

TABLE X

Spinnbarkeit in fertile patients.
(10 cases)

Sl. No.	Spinn- barkeit (cm)	Preovulatory phase		Ovulatory phase		Postovulatory Phase	
		No.of cases	Perce- ntage	No.of cases	Perce- ntage	No.of cases	Perce- ntage
1.	0 - 2	2	20.00	-	-	1	10.00
2.	2 - 4	6	60.00	-	-	7	70.00
3.	4 - 6	2	20.00	-	-	2	20.00
4.0	6 - 8	-	-	3	30.00	-	-
5.	8 - 10	-	-	4	40.00	-	-
6.	10 - 12	-	-	3	30.00	-	-
TOTAL		10	100.00	10	100.00	10	100.00

Table X shows spinnbarkeit in fertile group i.e. control group. It indicates that in preovulatory phase maximum number of cases (6, 60%) had length of cervical mucus i.e. spinnbarkeit in 2-4 cm range followed by 2(20%) cases in both 0-2 cm and 4-6 cm group.

Table also shows that in ovulatory phase maximum number of patients (4, 40%) had spinnbarkeit in 8-10 cm range while 3(30%) cases had 6-8 cm and 10-12 cm .

In most ovulatory phase maximum number of cases (7, 70%) had length of cervical mucus in the range of 2-4 cm, followed by 2(20%) cases in range of 4-6 cm and only one (10%) case had it in 0-2 cm range.

TABLE XI

Chloride content (m eq/l) in cervical mucus.
(fertile patients)

Sl. No.	Chloride content (m eq/l)	Preovulatory phase		Ovulatory phase		Postovulatory phase	
		No. of cases	Perce- ntage	No. of cases	Perce- ntage	No. of cases	Perce- ntage
1.	0- 50	-	-	-	-	-	-
2.	50-100	10	100.00	-	-	10	100.00
3.	100-150	-	-	4	40.00	-	-
4.	150-200	-	-	6	60.00	-	-
TOTAL		10		10		10	

It is evident from table XI that chloride content in cervical mucus in fertile patients varies with phase of menstrual cycle. In preovulatory phase all 10(100%) patients had chloride content in the range of 50-100 m eq/l.

In ovulatory phase, 6(60%) cases had chloride content in cervical mucus in the range of 150-200 m eq/l while rest 4(40%) cases had in the range of 100-150 m eq/l.

In post ovulatory phase again all 10(100%) cases had chloride content in the range of 50-100 m eq/l.

All patients of fertile group had secretory type of endometrium when endometrial biopsy taken in post ovulatory phase.

TABLE XII

Histopathology of endometrial biopsy taken
in post ovulatory phase (fertile patients).

(Total No.of cases 10).

Sl. No.	Histopathology of endometrial biopsy	No.of cases	Percentage
1.	Secretory	10	100.00
2.	Proliferative	0	00.00
	TOTAL	10	100.00

As regards the endometrial histology in control group (fertile group) is concerned, all patients had secretory type of endometrium when taken in post-ovulatory phase.

Forty infertile patients studied in three i.e. preovulatory, ovulatory and post ovulatory phases of cycle for spinnbarkeit and chloride content in cervical mucus. From various tables it has been seen that maximum cases 19(47.5%) were in the age group of 21-25 years (Table I) with maximum period of infertility 2-4 years (Table II).

Maximum patients, 24(60%) had length of cervical mucus i.e. spinnbarkeit below 5 cm in preovulatory phase (Table III) while in ovulatory phase length increased upto 10 cm (Table IV). Again in post ovulatory phase it was below 5 cm in 18(45%) cases (Table V).

Chloride level in cervical mucus was below 100 m eq/l between 7^m to 10^m day of the menstrual cycle i.e. in preovulatory phase in 33 (82.5%) cases. Between the 13^m to 16^m day of cycle i.e. in ovulatory phase majority of cases 26(65%) had chloride level between 100-150 m eq/l Twelve (30%) cases had chloride level between 150-200 m eq/l (Table VII). The highest value recorded was 178 m eq/l in one case. The chloride levels were below 100 m eq/l in majority of patients (24, 60%) between 16^m to 22^m day of cycle i.e. post ovulatory phase (Table VIII).

Majority of patients 37(92.5%) had secretory endometrium, biopsy taken in post ovulatory phase.

Only 3(7.5%) cases had proliferative phase (Table IX).

Total 10 fertile patients were studied. In fertile cases, in preovulatory phase, length of cervical mucus on stretching i.e. spinnbarkeit was upto 4 cm in 6(60%) cases while in ovulatory phase length was upto 10 cm in 4(40%) cases. In most ovulatory phase again length of cervical mucus on stretching was upto 4 cm in maximum number of cases i.e. 7(70%) (Table X).

All of fertile patients (100%) showed chloride levels below 100 m eq/l in preovulatory phase of cycle. However, it was 150-200 m eq/l in majority of patients 6(60%) during ovulatory phase. Again all had chloride content below 100 m eq/l in postovulatory phase (Table XI).

All fertile women had secretory type of endometrium on endometrial biopsy taken in postovulatory phase (Table XII).

D I S C U S S I O N

DISCUSSION

Many laboratory methods for detection and diagnosis of ovulation have been devised. Still this important basic phenomenon remains retrospective and numerous approaches have been realised to attest to its probable occurrence. The absolute confirmation of ovulation, though of profound biological significance has to be done by use of indirect means for want of simple direct one to establish such temporal relationship. The obvious significance in the detection and timing of ovulation is in the management of a case of infertile couple and greater importance for applying to the problem of fertility control.

Cervical mucus displays cyclical activity which is maximal at ovulation to facilitate migration, capacitation and the storage of spermatozoa in the cervical crypts. Many analyses, although useful, cannot routinely monitor the fertility period and establish probable ovulation dates.

The spinnbarkeit test and chloride estimation in cervical mucus are significant tests in relation to ovulatory phenomenon.

The present study showed a good correlation between spinnbarkeit test and chloride contents in cervical mucus with secretory endometrium in ovulatory cycles.

In normal fertile females there are cyclical changes in cervix under the influence of hormones.

The glandular elements of the cervix proliferate during the follicular phase and the epithelial cells become taller. Under the influence of oestrogen the glands also actively secrete a mucus which was stretch into threads measuring more than 6.5 cm and even 10-15 cm at the time of ovulation. This property of spinnbarkeit is the basis of the thread test for oestrogen in circulation.

During follicular phase the cervical mucus absorbs water and salts of sodium chloride and potassium chloride in a characteristic pattern which suggested the frond of fern.

At the time of ovulation the secretion is so profuse that it may be noticeable as a vaginal discharge. The ovulation cascade, its special character at this time makes for its easy penetration by spermatozoa. This property is related to its low content of protein and high contents of sugar.

During the luteal phase the cervical glands become more branched and their secretion changes its physical and chemical properties. The mucus becomes more viscous and form a more severe cervical plug. It loses its ability to stretch without breaking and resists penetration by spermatozoa. These changes are brought about by progesterone and are related to an increase in the amount of protein in the mucus and to the presence of phospholipids. Progesterone also reduces the electrolyte content of the mucus so by the twenty second day of the cycle its property of ferning or arborization on drying is lost.

The present study is based on the observations that forty infertile patients of reproductive age group (18-35 years) tested for spinbarkeit test and chloride content in cervical mucus to detect ovulation and results

were compared with endometrial biopsy in pre-menstrual phase.

As regard the spinnbarkeit phenomenon, a significant increase in length of cervical mucus was observed during ovulatory phase i.e. 10 cm or more. This finding was consistent with finding of Clift, Irving and Bernard (1958), Gibbon and Mathur (1966); Mitra et al (1975), Shammi Mehra and Eduljee (1978).

According to Clift, there was definite relationship between quantity, viscosity and spinnbarkeit of cervical mucus. Our studies corroborate his findings.

Irving and Bernard (1958) found that spinnbarkeit varied from 0-1 cm in preovulatory and post-ovulatory phases and 10-20 cm in ovulatory phase. From most reported series, spinnbarkeit at mid cycle exceeded 10 cm (Gibbon and Mathur, 1966 and Mitra et al, 1975).

Mehra and Eduljee (1978) reported spinnbarkeit ranged from 7.2 to 14.3 cm and where it fell below 6 cm post coital test was unfavourable.

In the present study spinnbarkeit varied from 1.2 cm to 6.0 cm in pre-ovulatory phase. In ovulatory

phase it was from 7.2 to 12.5 cm i.e. more than 6 cm.

In post ovulatory phase it ranged from 2 to 9.2 cm.

Significant rise was seen in chloride content in cervical mucus during ovulatory phase. Our findings were similar to previous studies done by Zondek and Rozin (1954), McSweeney and Sbarra (1964), Engineer et al (1968), Hardy et al (1970), Sarin (1971), Singh and Boss (1973), Campos da Paz (1974) and Mathur et al(1987).

Zondek and Rozin (1954) found that concentration of sodium chloride per unit weight of fresh mucus fluctuated cyclically in the menstrual cycle. McSweeney and Sbarra (1964) found chloride concentration in cervical mucus by spot test. They noted the result by the intensity of the spot or stain on the test paper ranged from 0.1% in early proliferative phase to a high value of about 0.9% at the presumable time of ovulation. The day after ovulation when the corpus luteum was producing progesterone, there was a decided change in spot intensity with a drop to below 0.5% in chloride concentration in the cervical mucus. This drop in sodium chloride concentration occurs simultaneously with the rise in temperature when using the basal temperature

chart. They presumed that ovulation takes place on the day or days of highest concentration of chloride in the cervical mucus. If there is little or no change in the spot test throughout the cycle, they regarded it there has been no ovulation.

Engineer et al (1968) and Sarin (1971) reported the correlation of cervical mucus in 99.1% and 84.5% respectively with endometrial biopsy.

Hardy et al (1970) found maximum chloride level on the day of ovulation as evidenced by brightest positive chloride spot on the test paper, and found that the mean concentration of sodium in fresh mucus varied from 72 to 95 micro mole/gram. Sodium(107 to 196 m mol/g) occurred on the day of ovulation and was usually preceded by a surge from a relatively low value 1 to 2 days prior to ovulation.

Singh and Boss (1973) found sodium chloride level to be constant at 100 to 150 m mol/kg throughout the cycle. However, in dry residue there were cyclical variation in the level with very sharp rise during the mid cycle.

Compos da Paz (1974) in his study also reported a complete correlation between caramel test fern pattern and endometrial biopsy.

Mathur et al (1978) found that chloride levels to be below 100 m eq/l between 7th to 12th day and after 17 day of menstrual cycle in majority of cases. Between 13th to 16th day of cycle i.e. on presumed days of ovulation the levels were above 100 m eq/l in ovulatory cycles. However, it was low in an ovulatory cycles.

In present study our findings were quite close to other's observations. Chloride levels in cervical mucus in pre-ovulatory phase ranged from 46 to 124 m eq/l while maximum number of patients had it in the range of 50-100 m eq/l.

In ovulatory phase chloride ranged between 90 to 184 m eq/l but majority of patients had chloride from 100-150 m eq/l. In post ovulatory phase it ranged from 45 to 118 m eq/l. Majority of patients had below 100 m eq/l chloride level.

The accuracy of cervical mucus chloride content and spinnbarkeit with endometrial biopsy was compared. In the present study it was found that the cervical mucus chloride estimation test to be fairly accurate when taken in consideration along with spinnbarkeit test as a means to assess the time of ovulation in fertile and infertile patients.

The findings of high levels of cervical mucus chloride in ovulatory phase was further confirmed by finding secretory endometrium on histopathology done in pre-menstrual phase of cycle. Ovulation was confirmed histologically in approximately 92.50% cases. Whereas patients who had an ovulatory cycles their cervical mucus chloride content was also low in mid cycle phase, justified that no ovulation occurred.

So estimating cervical mucus chloride as well as spinnbarkeit test is considering to be fair accurate as a means to be the ovulation as confirmed to endometrial biopsy.

C O N C L U S I O N

CONCLUSION

The present study was carried out to detect the ovulation by estimating the chloride contents in cervical mucus along with spinnbarkeit test in reproductive age group of infertile and fertile females. Results were compared with endometrial biopsy in premenstrual period.

In all a total of 50 cases were studied. These were divided into two groups. First group was study group consisting of forty infertile females and second was control group comprising of 10 fertile females.

Patients of study as well as control group were subjected for spinnbarkeit test and chloride estimation in cervical mucus three times in same menstrual cycle i.e. in preovulatory phase(7th to 12th day), ovulatory phase(13th to 16th day) and post-ovulatory phase (17th to 22nd day). Lastly endometrial biopsy was also taken in post-ovulatory phase.

Majority of patients showed significant increase in length of cervical mucus during ovulatory

phase along with significant rise in chloride contents as compared to their pre and post ovulatory phases. All these patients had secretory type of endometrium in premenstrual period.

While some cases did not show any significant increase in length of cervical mucus. There was no significant rise in chloride content also in cervical mucus during ovulatory phase as compared to pre and post ovulatory phase. These patients had proliferative type of endometrium.

It was concluded from the present study that physical and chemical characteristics of cervical mucus changed cyclically throughout the menstrual cycle.

Hence it can be concluded that chloride estimation in cervical mucus and spinnbarkeit test runs parallel with endometrial biopsy histopathologically as far as ovulation is concerned.

The Spinnbarkeit test and estimation of chloride levels in cervical mucus are fairly inexpensive and simple method for detection of

ovulation and in absence of facilities for proper histopathological studies which are invasive and other sophisticated tests.

Furthermore these simple tests can be utilised in the diagnosis and management of infertility cases. Apart from this knowledge about fertile period used by couples to avoid the fertile period in family planning methods.

S U M M A R Y

S U M M A R Y

In an attempt to detect ovulation by estimation of cervical mucus chloride levels, cervical mucus chloride estimation and spinnbarkeit test were done at least thrice(first before the 12th day, second at mid cycle and third in premenstrual phase) in the same cycle. Premenstrual endometrial biopsy was taken as a standard parameter for detection of ovulation.

Chloride levels in cervical mucus were found to be below 100 m eq/l between 7^m to 12^m day and after 22^m day of the cycle. Between 13^m to 16^m day of cycle i.e. presumed days of ovulation the levels increased above 200 m eq/l in ovulatory cycles. Levels remained low in an ovulatory cycles.

A positive correlation was observed between chloride levels and length of spinnbarkeit test. Length of spinnbarkeit was found below 5 cm in pre as well as postovulatory phases while in ovulatory phase length increased upto 10 cm.

Ovulatory cycles were detected in 95% cases by chloride levels and by length of spinnbarkeit test.

B I B L I O G R A P H Y

B I B L I O G R A P H Y

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A P P E N D I X

WORKING PROFORMADETECTION OF OVULATION BY CHLORIDE ESTIMATION IN CERVICAL
MUCUSCase No.

Name of Patient

Age

Chief Complaints

Menstrual History

P/V Findings :

Husband's Seminogram :

Pathological Datas :

I. Pre-ovulatory ($7^{\text{m}}-12^{\text{m}}$ day)

- a. Spinnbarkeit
- b. Chloride content in cervical mucus

II. Ovulatory ($13^{\text{m}}-16^{\text{m}}$ day)

- a. Spinnbarkeit
- b. Chloride content in cervical mucus

III. Post ovulatory ($18^{\text{m}} - 22^{\text{m}}$ day)

- a. Spinnbarkeit
- b. Chloride content in cervical mucus
- c. Endometrial biopsy